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GRANT NUMBER DAMD17-98-1-8589

TITLE: Relation of Genes to Prostate Cancer Occurrence and Aggressiveness

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REPORT DATE: July 1999

TYPE OF REPORT: Annual

PREPARED FOR: Commanding General
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 1999	Annual (1 Jul 98 - 30 Jun 99)

4. TITLE AND SUBTITLE
Relation of Genes to Prostate Cancer Occurrence and Aggressiveness

5. FUNDING NUMBERS
DAMD17-98-1-8589

6. AUTHOR(S)
John S. Witte, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Case Western Reserve University
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

We are studying brothers with prostate cancer in order to investigate the potential relation between genetic factors and their disease. The brothers are being recruited into the study from registries at Case Western Reserve University, the Cleveland Clinic Foundation, and the Henry Ford Health System. We are contacting men who have been diagnosed with prostate cancer at these institutions to see if they have brothers and whether they would be willing to join our study. In the first year of the study we have contacted 723 men diagnosed with prostate cancer who were eligible for taking part in the study. We have successfully recruited 519 of these men into the (72 %) have been recruited into the study. This recruitment rate is close to our expected level. While recruitment progresses, we have also detected a positive association between a genetic polymorphism and prostate cancer aggressiveness among African-Americans. This work has led to a paper provisionally accepted for publication, which is attached in the Appendix. The corresponding result implicates such genes in the development and/or aggressiveness of prostate cancer.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Prostate Cancer	25
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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John S. Wiff 7/26/99

PI - Signature Date

4. Table of Contents

5. Introduction

Prostate cancer is the most common non-skin cancer among men in the United States. This disease appears to run in families; many men who have close relatives with prostate cancer are at an increased risk of developing this disease themselves. Furthermore, such men might respond differently to treatment than men without a family history of prostate cancer. One possible explanation for the familiality of prostate cancer is genetics, and recent research has suggested some regions in DNA that might harbor alterations that increase the risk. We are studying brothers with prostate cancer in order to investigate the potential relation between genetic factors and their disease. The brothers are being recruited into the study from registries at Case Western Reserve University, the Cleveland Clinic Foundation (both in Cleveland), and the Henry Ford Health System (in Detroit). More specifically, we are contacting men who have been diagnosed with prostate cancer at these institutions to see if they have brothers and whether they would be willing to join our study. Since we are focusing on genetic factors, we are only contact those men in the registries who were aged 70 or younger when they were diagnosed. Our goal was to recruit the equivalent of 200 pairs of brothers into the study. We are collecting blood from the men, as well as questionnaire information. From the blood we will get DNA, and then use this information look at those genes that plausibly could lead to prostate cancer, based on current scientific evidence. In particular, we are studying whether the brothers with prostate cancer inherit certain forms of the genes more often than expected by chance, and whether such forms of these genes are associated with the aggressiveness of prostate cancer. If so, this implicates such genes in the development and/or aggressiveness of prostate cancer. The information from this study will help provide men with additional knowledge about their risk of prostate cancer and, if they are already diseased, how genes might influence their response to treatment.

6. Body

Below we describe out research accomplishments with respect to the original Statement of Work. For focus and clarity we reproduce the approved tasks that were proposed for completion by the end of year 1. We then note our corresponding accomplishments, pointing out any issues arising during the course of this work.

Task 1. ***Prepare for subject recruitment:***

- ***a. Develop and test family history and risk factor questionnaires.***
- ***b. Train coordinators to administer the telephone questionnaires.***
- ***c. Obtain Institutional Review Board approval from all collaborating institutions.***
- ***d. Create database for storing information on study subjects.***

We have successfully accomplished Task 1, which was initiated under a small pilot grant. Specifically, we have developed and tested the questionnaires, trained coordinators to administer the questionnaires, and received Institutional Review Board approval from all collaborating institutions. Finally, we have created Microsoft Access databases for storing recruitment and questionnaire information.

Task 2. ***Ascertain probands and determine family history:***

- ***a. Determine contact information from our registries for approximately 1500 potential probands (i.e., index cases with prostate cancer).***
- ***b. Send initial letter describing the study to these men.***
- ***c. Follow-up with a phone interview requesting family history information, and obtain consent for inclusion in the study.***

Task 2 was also initiated under the small pilot grant. Here we have been successful in building a database that contains contact information from our registries for 3000 potential probands, twice the number initially proposed. To date, we have sent an initial letter describing the study to over one-third of these men, and then contacted them to determine their eligibility / willingness to take part in the study. (Below we give more details on subject recruitment.)

Task 3. ***Recruit probands' brothers into study:***

- ***a. Send letter describing the study to approximately 300 men with prostate cancer, noting their brother's (i.e., the proband's) willingness to participate.***
- ***b. Follow-up with a phone call to determine whether they are agreeable to taking part in the study, and get consent from these (non-proband) subjects.***

Once a proband was deemed to be eligible for taking part in the study, and indicated a willingness to join in our research project (per Task 2), we sent introductory letters to their brothers. This has been successfully followed with recruitment phone calls to enroll them into the study with their consent. One difficulty faced here has been the detection of brothers who also have prostate cancer. That is, finding brothers where both have prostate cancer is going slower than anticipated. (Our original proposal pointed this out as a potential limitation.) To help deal with this issue, we have expanded our recruitment in two ways. First, as suggested in our original application, we are now including those brothers either with or without prostate cancer. For the non-diseased brothers, to address

potential bias issues, we are restricting recruitment to those who are either older siblings, or if younger, within at least 10 years of the cases age at diagnosis. Second, we have slightly relaxed our age cutoff for inclusion in the study to 70 years old at diagnosis (the original cutoff was 65 years). A cutoff was chosen to restrict our subjects to men most likely harboring genes involved with prostate cancer. Shifting this up by 5 years should not have much impact on our ability to evaluate genetic factors involved with prostate cancer, especially since it appears that much prostate cancer might arise from high frequency, low penetrance genes common among men of all ages.

Task 4. ***Collect blood and questionnaire information on ~400 subjects (200 pairs):***

- ***a. Obtain and store blood samples.***
- ***b. Undertake phone risk factor interviews, and enter information into database.***
- ***c. Write up year 1 report of study progress.***

We have successfully collected and stored blood and questionnaire information from 519 subjects, arising from 291 sibling sets. For these sets, some contain more than one brother. Ninety-nine of these sets only include a single sibling at the present time (we are currently bringing the corresponding siblings into the study). Over 50% of the sibling sets constitute brothers discordant for prostate cancer. Since discordant brothers will likely be less statistically powerful than concordant brothers, we are continuing our recruitment into year 2, and will ultimately have well beyond the initially proposed 200 pairs. In addition, we are now collaborating with another sib-pair study (PI William Catalona) to increase the total number of concordant sib-pairs available for analysis. Sib-pairs collected here are presently being genotyped for a genome-wide scan in conjunction with sib-pairs Dr. Catalona has collected over the past decade. This effort should result in more power than otherwise available to us, and hence potentially more promising scientific results.

While recruitment was ongoing during year 1, we have also evaluated the relation between a recently reported polymorphism in the CYP3A4 gene and prostate cancer aggressiveness among Africa-Americans. Our results indicate that the polymorphism is positively associated with aggressiveness at presentation, as measured by tumor stage and grade. This work has been accepted for publication in *Cancer Epidemiology, Biomarkers, and Prevention*, and is included here as Appendix 2.

7. Key Research Accomplishments

During the initial year of this grant we have produced the following key research accomplishments.

- Developed and tested study questionnaire.
- Hired and trained study coordinators for the project.
- Obtained Institutional Review Board approval, and annual renewals, from all collaborating institutions.
- Created Microsoft Access databases for storing information on study subjects. One database is for recruitment purposes only, and tracks subject's progress through phases of recruitment. Another database stores questionnaire information, and does not have any identifying information.
- Built up recruitment database to include contact information for over 3000 potential probands (i.e., index cases with prostate cancer).
- Tested and instigated recruitment process for probands and their brothers, whereby initial letters describing the study are followed by phone interviews and/or in person interviews.
- Fully recruited 519 men into the study. That is, we have collected and stored consent, blood, and questionnaire information on these men.
- Undertaken near-term research looking at relation between a genetic polymorphism and prostate cancer aggressiveness in African-American men. This work indicates that this polymorphism may be involved with aggressiveness, and has been accepted for publication (Appendix).
- Became involved with a collaborative effort to map genes for prostate cancer development and aggressiveness.

8. Reportable Outcomes

To date, our reportable outcomes include the following manuscript and presentations.

- Paris PL, Kupelian PA, Hall JM, Williams TL, Levin H, Klein EA, Casey GC, Witte JS. "Association between a CYP3A4 genetic variant and clinical presentation in African American prostate cancer patients." Cancer Epidemiology, Biomarkers, and Prevention 1999, provisionally accepted.
- "Prostate Cancer Genetics Study (CaP Genes)." Prostate Cancer Research Group, Case Western Reserve University, Summer 1998.
- "CaP Genes Study: Overview and progress." Cleveland Clinic Foundation Prostate Cancer Retreat, Fall 1998.
- "Prostate cancer genetic epidemiology." Mayo Clinic, Rochester, Winter 1998.
- "Prostate Cancer Genetics." Man-to-Man, Cleveland, Spring 1999.

9. Conclusions

During the first year of this project we have successfully launched our prostate cancer genetics study. In particular, we have set up and carried out an efficient program for recruiting a large number of brothers into the study. This is important because recruiting a sufficient sample size is critical for the successful completion of the scientific aims of the project. We have attempted to address issues surrounding sample size limitations by extending our recruitment to include older men, non-diseased brothers, and will continue recruiting men well into the second year of the study. While recruitment was underway, we also undertook a project to look for the relation of a genetic polymorphism and prostate cancer aggressiveness among African-Americans. Our findings indicated a positive association between a common single nucleotide poolymorphism in the CYP3A4 gene and tumore grade / stage (Appendix). The value of this knowledge—and the information eventually gleaned from the rest of this project—as a scientific or medical product lies in our ability to use such information to predict which individuals may need more or less serious treatment following diagnosis with prostate cancer. In particular, if men with the observed polymorphism are indeed at an increased risk of prostate cancer aggressiveness, then information about the polymorphism could be used for deciphering the optimal course of clinical treatment.

10. References

Paris PL, Kupelian PA, Hall JM, Williams TL, Levin H, Klein EA, Casey GC, Witte JS. "Association between a CYP3A4 genetic variant and clinical presentation in African American prostate cancer patients." Cancer Epidemiology, Biomarkers, and Prevention 1999, provisionally accepted.

Appendix

Association between a CYP3A4 genetic variant and clinical presentation in African American prostate cancer patients[1]

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[1] This work supported in part by grants from the US Army (DAMD17-98-1-8589), the General Motors Foundation, and the UH/CWRU Ireland Cancer Center.

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Running title: CYP3A4 polymorphism and prostate cancer

Abstract

Prostate cancer incidence, clinical presentation, and mortality rates vary among different ethnic groups. A genetic variant of CYP3A4, a gene involved in the oxidative deactivation of testosterone, has recently been associated with prostate cancer development in Caucasians. To further investigate this variant, we evaluated its genotype frequencies in different ethnic groups, and its association with clinical presentation of prostate cancer in African Americans.

CYP3A4 genotypes were assayed in healthy male Caucasian (N = 117), Hispanic (N = 121), African American (N = 116), Chinese (N = 46), and Japanese (N = 34) volunteers using the TaqMan assay. The association between CYP3A4 genotype and prostate cancer presentation was determined in 174 affected African American men.

Genotype frequency of the CYP3A4 variant differed substantially across ethnic groups, with African Americans much more likely to carry one or two copies than any other group (two-sided $P < 0.0001$). Among African Americans, 46 % (80 of 174) of men with prostate cancer were homozygous for the CYP3A4 variant, whereas only 28 % (32 of 116) of African American healthy volunteers were homozygous (two-sided $P < 0.005$). A consistent positive association was observed between being homozygous for the CYP3A4 variant in African American prostate cancer patients and clinical characteristics. Men homozygous for the CYP3A4 variant were more likely to present with higher grade and stage of prostate cancer in a recessive model (OR = 1.7; 95 % CI = 0.9 - 3.4). This association was even stronger for men who were over 65 years old at diagnosis (N = 103; OR = 2.4; 95 % CI = 1.1 - 5.4).

In summary, the CYP3A4 genotype frequency in different ethnic groups broadly followed trends in prostate cancer incidence, presentation and mortality in the U.S. African American prostate cancer patients had a higher frequency of being homozygous for the CYP3A4 variant than healthy African American volunteers who were matched solely based on ethnicity. Among the patients, those who were homozygous for the CYP3A4 variant were more likely to present with clinically more advanced prostate cancer.

Introduction

Prostate cancer is the most common non-skin related cancer affecting men in the United States and the second leading cause of cancer related deaths (1). The incidence of prostate cancer varies substantially across ethnic groups, with African American men exhibiting the highest rates worldwide, and Asian men having the lowest rates (2). Furthermore, African American men generally present with more severe forms of prostate cancer, potentially leading to a more aggressive course of the disease (3,4). Unfortunately, the etiology of prostate cancer remains unclear, with little known about molecular markers that may help distinguish between an indolent versus an aggressive clinical course. The identification of genetic or molecular risk factors for prostate cancer susceptibility and aggressiveness, including those that distinguish ethnic differences, is an important goal.

Testosterone plays a critical role in stimulating prostate cell division. CYP3A4, a protein belonging to the cytochrome P450 supergene family, is involved in the metabolism and most likely the deactivation of testosterone (5,6). A germline genetic variant in the 5' regulatory region of the CYP3A4 gene (A to G transition at position minus 293 from the ATG start site) has recently been reported and was found to be associated with a higher clinical grade and stage in Caucasian men with prostate cancer, especially among those diagnosed at a later age with no family history of the disease (7). Therefore, we examined the genotype frequencies of the CYP3A4 variant in different ethnic groups, and determined that the variant was much more common in African Americans. This motivated us to undertake a study of African Americans that had been diagnosed with prostate cancer, in order to evaluate whether the presence of the CYP3A4 variant was associated with clinical characteristics (Gleason grade, prostate specific antigen (PSA), and tumor-lymph node-metastasis (TNM) stage) that play a role in the clinical course of this disease.

Materials and Methods

Subjects

To determine the CYP3A4 allele frequencies across ethnic groups, a convenience sample of 117 Caucasian, 121 Hispanic, 116 African American, 46 Chinese, and 34 Japanese healthy male volunteers from the Southern California area were enrolled in the study. A blood sample was collected from each subject who self reported ethnicity and medical status following signed consent. A sample of 205 African American men diagnosed with prostate cancer between years 1993 and 1998 at the Cleveland Clinic Foundation were identified through the Cleveland Clinic Foundation's Familial Cancer Registry. Of the 205 tissue samples for which tissue blocks or slides were available, 174 yielded DNA of sufficient quality for PCR amplification and were included in the study. All prostate cancer samples were either biopsies at the time of diagnosis or resections at the time of surgery. The study design was approved by the IRB of the Cleveland Clinic. Clinical characteristics, including Gleason grade, PSA, and TNM stage, as well as other potentially important factors, such as age at diagnosis and family history of prostate cancer, were obtained from medical records. Family history, a question that was routinely asked as part of an office visit, was defined as having at least one first degree relative with prostate cancer. Tumor grade was determined according to the Gleason system (8). The tumor stage was determined after review of the microscopic sections of the specimen (9). Among prostate cancer patients, the mean age at diagnosis was 66 years, with a range of 43 to 91 years.

DNA extraction

For healthy volunteers, DNA was extracted from blood using a kit from Gentra Systems, Inc. (Minnesota). For prostate cancer cases, DNA was extracted from sectioned paraffin-embedded tissue blocks (ten 10 micron sections) or pathology slides. Paraffin was removed following treatment with xylene. DNA was then extracted using the QiaAmp Tissue Kit (QIAGEN, California). The final elution was in 50 μL or 100 μL Tris pH 9 buffer for slides or sections, respectively.

CYP3A4 Variant Detection by the TaqMan Assay

The CYP3A4 genotype was determined using the TaqMan assay (10). Samples were assayed in triplicate in a Robbins 96-well plate. The primers for CYP3A4 were derived from

published sequence (11). A 126 bp fragment was amplified by PCR in reactions containing 20 ng genomic DNA, 900 nM forward unlabeled inner primer (5'-ATCTGTAGGTGTGGCTTGTTGG-3'), 900 nM reverse unlabeled inner primer (5'-TATCAGAAACTCAAGTGGAGCCAT-3'), 200 nM FAM labeled probe (5'-TTAAATCGCCTCTCTCTTGCCCTTGTCTCTAT-3'), 200 nM TET labeled probe (5'-AATCGCCTCTCTCCTGCCCTTGTCTCTAT-3') and 1X Perkin-Elmer TaqMan Reagent Mix #43C4447. PCR reactions were preincubated at 50 °C for 2 minutes, then 95 °C for 10 minutes. Two-step thermocycling was performed for 40 cycles: denaturation at 94 °C for 30 seconds and annealing at 60 °C for 30 seconds. Upon completion of thermocycling, the fluorescence was read on an ABI 7700 Sequence Detector using the allelic discrimination software. FAM to TET ratios for each sample DNA, normalized against the TAMRA signal, indicated the CYP3A4 promoter genotype of each patient and was further confirmed by similar signals from the known control DNAs.

For prostate cancer patients, the CYP3A4 genotype was determined by the TaqMan assay following a nested PCR amplification and DNA quantitation, using DNA extracted from paraffin. A 297 bp fragment containing the CYP3A4 promoter region was amplified by PCR using the following outer nested primers and conditions: unlabeled forward 5'-GCTCTGTCTGTCTGGGTTTGG-3' and unlabeled reverse 5'-CACACCACTCACTGACCTCCT-3': 33.5 mM Tris-HCl, pH 8, 8.3 mM $(NH_4)SO_4$, 25 mM KCl, 2.5 mM $MgCl_2$, 0.85 mg/mL BSA, 0.25 mM each dNTP, 0.015 U AmpliTaqGold per 20 μL PCR reaction, and 10 to 15 μL of eluted DNA (concentration unknown). Touchdown thermocycle conditions were used: 95 °C for 10 minutes; 3-cycle PCR 94 °C for 30 seconds, 66 °C for 30 seconds, 72 °C for 30 seconds decreasing 1 °C per cycle for 16 cycles; then 3-cycle PCR 94 °C for 30 seconds, 50 °C for 30 seconds, 72 °C for 30 seconds for 22 cycles; 72 °C for 4 minutes; 4 °C hold.

Concentrations of various dilutions of the resultant PCR products containing pico green were measured on a CytoFluor II spectrophotometer against a standard curve of known

concentrations of human placental DNA. Five nanograms of DNA were used for each PCR reaction using the inner nested primers in the TaqMan assay described above. Genomic DNAs containing known CYP3A4 genotypes were processed in the same manner as controls for the assay. The control DNAs included one homozygous wild type (AA), one heterozygous (AG) and one homozygous (GG) variant sample (confirmed by DNA sequencing).

A randomly selected homozygous variant sample (concluded from the TaqMan assay) was directly sequenced. Sequencing was carried out by the Molecular Biotechnology Core within the Lerner Research Institute using an ABI 377 DNA Sequencer (ABI, California). The sample was shown to carry the expected nucleotide change, an A to G transition in the 5' regulatory region of the CYP3A4 gene.

Statistical Methods

CYP3A4 genotype frequencies were calculated within each ethnic group and among the men with prostate cancer, using data obtained with the TaqMan assay. To compare these observed frequencies with their expected values across ethnic groups, and between African American volunteers and prostate cancer patients, Pearson χ^2 test statistics were calculated. All corresponding P-values are two-sided. The frequencies, χ^2 tests, and P-values were all calculated using the GAUSS programming language (Aptech Systems, Inc., Washington).

For the comparison of clinical characteristics in the African American cases, odds ratios were calculated to estimate the relative risks that carriers of CYP3A4 variants present with more aggressive clinical characteristics. The CYP3A4 genotypes with one or two variants were investigated individually (*i.e.*, AG versus AA, GG versus AA), and in combinations that reflected recessive (GG versus AG and AA combined) and dominant (GG and AG combined versus AA) models. Categories of clinical characteristics were defined a priori as follows: Gleason grade, two groups (cutpoint = 7); PSA at diagnosis, two groups (cutpoint = 10 nanograms / microliter); TNM stage, two groups (T1a-c, T2a-b versus T2c, T3, T4, or metastatic). Following Rebbeck *et al.* (7), a constellation of grade and stage characteristics was defined as "low" (Gleason grade ≤ 7 and tumor stage T1a-c, T2a-b) and "high" (Gleason grade >

7 or tumor stage T2c, T3, T4, or metastatic). Odds ratios (OR) and 95% confidence intervals (CI) were calculated using logistic regression (SAS Institute Inc., North Carolina). These models included age at diagnosis (continuous variable) and family history (defined as the existence of any first-degree relative with prostate cancer) as potential confounders. The possible effect modification by these factors was also evaluated (per Rebbeck *et. al.*, 7) by undertaking analyses of the data stratified by age at diagnosis (cutpoint = 65 years). This latter analysis still included age at diagnosis (continuous) and family history as potential confounders. Additional stratification by family history was also performed. Finally, three subjects had some missing values due to incomplete medical records (*i.e.*, one was missing PSA, one TNM stage, and one PSA and TNM stage), and were excluded from the corresponding analyses.

Results

CYP3A4 frequencies among ethnic groups

Genotype frequencies of the CYP3A4 variant across ethnic groups are shown in Table 1. The G variant was not found in the Chinese or Japanese populations. At least one copy of the variant was identified in 7 % (8 of 117) and 20 % (24 of 121) of the Caucasian and Hispanic populations, respectively. In contrast, 81 % (94 of 116) of the African American population carried at least one variant allele, and 28 % (32 of 116) were GG homozygotes. Comparing the genotype frequency in African Americans to that in the other ethnic groups gave two-sided P values < 0.0001 for each comparison. During the review process, a relevant online paper was brought to our attention. In examining three ethnic groups, Walker et al. estimated the allele frequency of the CYP3A4 variant to be 0.53 in African Americans, 0.09 in Caucasians and 0 in Taiwanese (12). This is consistent with the trend that we found in African American, Caucasian, and Asian populations.

Association of CYP3A4 Genotype and Prostate Cancer

Among African Americans with prostate cancer, 83 % (144 of 174) carried at least one copy of the variant allele. Thirty seven percent (64 of 174) of those with a variant were

heterozygotes, whereas 46 % (80 of 174) were homozygous (Table 1). Comparing the CYP3A4 genotypes between these men and the African American healthy volunteers gave a two-sided P value = 0.005. Comparing the homozygous (GG) versus the AA and AG combined gave a two-sided P-value = 0.002 between these two groups.

Stratification of the CYP3A4 genotypes and clinical characteristics in the 174 African Americans with prostate cancer are shown in data columns 1 through 3 in Table 2. Fifty five percent (16 of 29) of the men presenting with a Gleason grade over 7 were homozygous for the variant, whereas only 44 % (64 of 145) presenting with a lower Gleason grade were homozygous for the variant. A similar difference was observed for the grade/stage variable. Forty nine percent of the men homozygous for the CYP3A4 variant presented with PSA > 10, while only 41 % with PSA $\leq$ 10 were homozygous. There was no difference in genotype by TNM stage.

The fourth column of Table 2 shows ORs comparing GG to AG and AA combined (reflecting a recessive model). In this case, being homozygous for the CYP3A4 variant appeared to increase the risk of presenting with high grade/stage (OR = 1.7; 95 % CI = 0.9 - 3.1). Slightly weaker associations were observed for Gleason grade (OR = 1.6) and PSA at diagnosis (OR = 1.6). When comparing GG and AG to AA (reflecting a dominant model) we found similar, albeit modest, positive associations. In particular, for high Gleason grade the OR = 2.1 (95% CI = 0.6 - 7.4), whereas for high grade/stage and PSA the OR =1.4 (95% CI = 0.5 to 3.5, and 0.6 - 3.4, respectively). A comparison of men with AG to AA, and GG to AA, showed a slight increasing trend for Gleason grade, where the adjusted ORs were 1.7 and 2.4, respectively. For PSA at diagnosis and grade/stage, there were only positive associations for the comparison of GG to AA, where ORs = 1.8 and 1.7, respectively. The 95 % CIs for these associations were, however, quite wide, reflecting the small number of subjects with some genotype/clinical characteristic combinations.

Data restricted to men over the age of 65 are shown in Table 3. In this group, approximately 10 to 20% more men presented with more severe clinical characteristics if they were homozygous for the variant. Looking at the recessive model, having two copies of the

CYP3A4 variant was associated with presenting with higher Gleason grade and PSA at diagnosis (ORs = 2.2) (column 4 of Table 3). As above, however, the CIs for both of these associations were somewhat wide (lower bounds = 0.9 and 0.8, respectively). We observed a stronger association for grade/stage (OR = 2.4; 95 % CI = 1.1 - 5.4). The dominant model (i.e. GG and AG versus AA), and a comparison of AG to AA, and GG to AA gave relatively weaker results (not shown). Additional stratification by family history did not show stronger results.

Discussion

In this study, we show that the frequency of a germline CYP3A4 variant is substantially higher among African American men than among other ethnic groups, and that African American men with prostate cancer have significantly higher frequency of being homozygous for the CYP3A4 variant than healthy African Americans. We report consistent positive associations between the CYP3A4 variant and clinical characteristics in African American men with prostate cancer. The strongest association was between homozygous variant carriers and high Gleason grade or grade/stage when restricted to men over 65 years. This finding is in agreement with a recent study reporting on the association between the CYP3A4 variant and prostate cancer in a Caucasian population (7). Therefore, the CYP3A4 variant may represent an important prognostic factor for prostate cancer occurrence and aggressiveness among not only Caucasians, but also African Americans. However, these data should be verified in a larger population. In addition, differences in the frequency of the variant between ethnic groups might help to explain some of the inter-ethnic differences in the incidence, presentation, and mortality of this disease.

The CYP3A4 variant reported is an A to G alteration that occurs in the nifedipine-specific element (NFSE) in the 5' regulatory region of the CYP3A4 gene. This element may be required for expression of the CYP3A4 gene (11). The CYP3A4 protein oxidizes testosterone, which might also deactivate the hormone, although this has yet to be proven (5,6). As a result, men carrying the CYP3A4 variant allele may have more testosterone available to be converted to dihydrotestosterone (DHT), which is the main male sex hormone that regulates prostate cell

division (13). Consequently, there exists a biological rationale for the CYP3A4 variant playing a role in prostate cancer development and aggressiveness. Metabolism of testosterone may no longer be efficiently processed in men carrying the CYP3A4 variant (especially in those homozygous for the variant), leading to increased prostate cell proliferation due to the increased bioavailability of testosterone. This higher bioavailability may be most important among older men, who due to the aging process have lower basal testosterone levels than younger men (7, 14). Furthermore, the statistically significant result for African American men with a later age at diagnosis is what one would expect for the CYP3A4 variant because it appears to have a high frequency but low penetrance. That is, many men carry the variant but only some present with more severe clinical characteristics. In contrast, men carrying an uncommon, but highly penetrant genetic mutation (for example HPC1) (15) will be more likely to have an early age of onset and a positive family history of prostate cancer.

The substantial difference in CYP3A4 variant genotype frequencies among the ethnic groups evaluated here was intriguing with regards to the potential involvement of this variant in prostate cancer development. Asians, who have the lowest rates of prostate cancer, were found not to carry the CYP3A4 variant. Some Hispanics and Caucasians, who have prostate cancer incidence rates between Asians and African Americans, carried the variant at a much lower frequency than the African American group. These data are consistent with previously reported findings (12). Therefore, the frequency of the CYP3A4 variant broadly parallels previously reported (2) incidence rates across ethnic groups in the U.S.

There are a number of limitations to this study. First, our comparison of genotype frequencies between African American prostate cancer patients and healthy volunteers only supports ecologic-level inferences. While appearing healthy at the time of blood collection, some of the volunteers may develop prostate cancer later in life. Nevertheless, we would expect this potential misclassification to be non-differential, and thus lead to an underestimate of our observed differences in CYP3A4 genotype frequencies between prostate cancer patients and healthy volunteers (16). A recent study, however, observed a similar allele frequency (0.53) in

the CYP3A4 variant among African Americans from Pennsylvania (12) as observed in our California African Americans (0.54). This suggests that any potential differences in racial admixture between California and Pennsylvania African Americans did not alter CYP3A4 frequencies, and by geographic extrapolation, that there may not be such differences between California and Ohio. If the variant is associated with prostate cancer incidence and/or survival, then it will be important to establish the allele frequencies across age groups in future studies. However, a carefully conducted case-control study will resolve these issues. Second, the associations observed in our evaluation of prostate cancer clinical characteristics were consistent but due to sample size limitations some had broad 95 % confidence intervals. While these associations were modest, the high frequency of the CYP3A4 variant and the increased incidence and mortality of prostate cancer among African Americans suggests an association between disease and the presence of the variant, particularly in homozygous carriers. Third, since most subjects in this study presented with clinically localized disease, we could only compare a moderate range of clinical characteristics, which did not allow for a full analysis of the impact of the CYP3A4 variant on prostate cancer aggressiveness. Finally, the consequences of carrying a particular CYP3A4 genotype may differ depending on the disease or outcome being investigated. In fact, possession of the wild type genotype has been recently associated with an increased risk for treatment-related (chemotherapy) leukemia (17).

In summary, African Americans have a higher incidence of prostate cancer and appear to present with more advanced stage disease at time of diagnosis (3,4). The data that we present on African American prostate cancer patients, along with other recent work (7), implies that carriers of the CYP3A4 variant are more susceptible to the development of more aggressive forms of this disease. If the association reported here is supported by further research and should prove to be indeed causal, early genetic screening for the CYP3A4 variant may be warranted, and post-diagnostic screening could prove useful in choosing the most appropriate course of treatment. However, it should be recognized that prostate cancer is a complex disease, and therefore this gene may only play a small part in its etiology.

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Table 1 Genotype frequencies of CYP3A4 wild type (A) and variant (G) in men, across ethnic groups and among African American healthy volunteers and prostate cancer patients

Group	N[a]	Genotype Frequency[b] AA	AG	GG	P-value[c]
Asian[d]	80	1.00	--	--	<0.0001
Caucasian	117	0.93	0.06	0.01	<0.0001
Hispanic	121	0.80	0.18	0.02	<0.0001
African-Americans					
Healthy volunteers	116	0.19	0.53	0.28	[c]
Prostate cancer patients	174	0.17	0.37	0.46	0.005
					0.002[e]

[a] N number of subjects

[b] A, wild type; G, variant.

[c] Two-sided P-values from Pearson χ^2 tests comparing frequencies in each group with frequencies in African American healthy volunteers. Among African Americans, P-value from a comparison of healthy volunteers and prostate cancer cases.

[d] Asian include Chinese American (N = 46) and Japanese Americans (N = 34).

[e] P-value comparing GG versus AA and AG combined.

Table 2 Association of CYP3A4 genotype and clinical characteristics among African American prostate cancer cases.

Clinical Characteristic	CYP3A4 genotype, N (%)[a] AA	AG	GG	Odds ratio (95 % CI)[b]
Gleason grade				
≤ 7	27 (19 %)	54 (37 %)	64 (44 %)	1.0
> 7	3 (10 %)	10 (34 %)	16 (55 %)	1.6 (0.7 - 3.6)
PSA at diagnosis[c]				
≤ 10	13 (20 %)	25 (39 %)	26 (41 %)	1.0
> 10	16 (15 %)	39 (36 %)	53 (49 %)	1.6 (0.8 - 3.1)
TNM Stage				
T1a-c/T2a-b	24 (17 %)	51 (37 %)	64 (46 %)	1.0
T2c/T3/T4/M	6 (17 %)	13 (37 %)	16 (46 %)	1.0 (0.5 - 2.2)
Grade / Stage[d]				
Low	23 (19 %)	48 (39 %)	53 (43 %)	1.0
High	7 (14 %)	16 (32 %)	27 (54 %)	1.7 (0.9 - 3.4)

[a] N number of subjects. A, wild type; G, variant.

[b] CI, confidence internal. Comparing those with GG versus those with AA and AG genotypes (recessive model). Adjusted for age at diagnosis and family history of prostate cancer.

[c] Units for PSA = nanogram / milliliter

[d] Combined Gleason grade and TNM stage is defined as: "Low" = T1a-T1c or T2a-b stage and Gleason grade ≤ 7; "High" = T2c, T3, T4 or M stage and Gleason grade > 7. Gleason grading is detailed in (8) and TNM staging in (9).

Table 3 Association of CYP3A4 genotype and clinical characteristics among 103 African American prostate cancer cases over the age of 65.

	CYP3A4 genotype, N (%)[a]			
Clinical Characteristic	AA	AG	GG	Odds ratio (95 % CI)[b]
Gleason grade				
≤ 7	15 (19 %)	35 (44 %)	30 (37 %)	
> 7	3 (13 %)	7 (30 %)	13 (57 %)	2.2 (0.9 - 5.8)
PSA at diagnosis[c]				
≤ 10	6 (24 %)	12 (48 %)	7 (28 %)	
> 10	11 (14 %)	30 (39 %)	36 (47 %)	2.2 (0.8 - 5.9)
TNM Stage				
T1a-c/T2a-b	12 (16 %)	33 (45 %)	29 (39 %)	
T2c/T3/T4/M	6 (21 %)	9 (31 %)	14 (48 %)	1.4 (0.6 - 3.3)
Grade/Stage[d]				
Low	11 (17 %)	31 (49 %)	21 (33 %)	
High	7 (17 %)	11 (28 %)	22 (55 %)	2.4 (1.1 - 5.4)

[a] N number of subjects. A, wild type; G, variant.

[b] Comparing those with GG versus those with AA and AG genotypes (recessive model). CI, confidence internal. Adjusted for age at diagnosis and family history.

[c] Units for PSA = nanogram / milliliter

[d] Combined Gleason grade and TNM stage is defined as: "Low" = T1a-T1c or T2a-b stage and Gleason grade ≤ 7; "High" = T2c, T3, T4 or M stage and Gleason grade > 7. Gleason grading is detailed in (8) and TNM staging in (9).